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Are "Multiple Cross-Multiple Pollen Hybrids" an Answer for Productive Populations in *Brassica campestris* var. 'brown sarson'?*

I. Methods for Studying 'Mucromphs'

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Summary. A set of complex crosses with multiple crosses as female parents were made using multiple pollen in turnip rape (*Brassica campestris* L.). These multiple cross – multiple pollen hybrids (mucromphs) were evaluated for a large number of quantitative characters including yield. New methods were proposed to study such genetic material in depth so as to formulate suitable strategies to breed for attractive seed yield.

Key words: Multiple cross – Combining ability – *Brassica campestris* – Genetic characterisation – Oil seed breeding

Introduction

Methods to study hybrids between homozygotes in mono – gene – diallelic systems are well developed and have been extended to a class of polygenic situations (Mather and Jinks 1971, 1977). The basic approach has been to develop theoretical concepts on well-defined genetic systems and then to enquire (through statistical methods) whether an actual experiment conforms to those systems. If it does, it is easy to extend the interpretations already available.

Modern concepts of plant breeding aim at genetic reconstruction with respect to a number of vital character components. A breeder is thus required to work with a broad genetic base of parents and hence hybrids. Rarely, if ever, can one hope to identify the genotypic structure one is working with to the required extent, though it is possible to have an apriori idea of the inclusion, or otherwise of the specific effects, of some genes like those related to dwarfing and disease resistance.

Scientific plant breeding is a stochastic process. The primary need of a plant breeder is an objective evaluation of the current material to decide which course of action to take in raising the next generation. In general, a relative evaluation of the material using measurable genetic effects is sufficient. Methods developed for such a purpose should have high repeatability though they may be of use only to rank the relative potential of the material.

Turnip rape (*Brassica campestris* L.) is an important oilseed crop of India. It has three major varietal forms – 'brown sarson', 'yellow sarson' and 'toria'. In 'brown sarson' different levels of self-incompatibility are also available. The crop being cross-pollinated, the advantage of providing for a broad genetic base in complex hybrids and producing high yielding derivatives in advanced generations weighed in favour of working for a strategy to produce desirable complex crosses. A novel attempt was made to choose a multiple cross female and to employ multiple pollen from chosen cultivars in order to obtain a desirable broad genetic base through multiple cross – multiple pollen hybrids (to be designated as mucromphs for short).

In its wake, several other problems, such as choice of parents, suitable mating systems, methods of hybridisation among panmictic parents, appropriate genetic models, efficient methods of inference and judicious choice of characters, cropped up anew.

Methods developed to answer these problems and the results on yield improvement of this crop will be discussed in this series of papers, the first of which deals with the methods of studying mucromphs.

Material and Methods

The female parents were chosen to represent a diverse genetic base and included single cross (SC) and three-way (TC) crosses as well as elite varieties (VT) and biparental progenies (BP). In SC, potential crosses between lines derived from disruptive and stabilising

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selection (5995 and 1555) and inter-varietal crosses (KLIB) were included (Table 1). Intra-line variants in some disruptive selection lines were used to provide Productive recombinant (PV) X Original (OR) and their reciprocal crosses. They were mated in their F1 stage to some promising cultivars to provide TC (Arunachalam and Katiyar 1978). Potential TC in their F2 constituted four female parents of mucromphs. Biparental matings in some inter-varietal crosses (Amirthadevarathinam et al. 1976) were advanced to their F2 generation and chosen single plant progenies were also included as parents in addition to three promising cultivars, two of which were exotic (Table 1).

Pollen was composited from 3 exotic and 5 indigenous cultivars (Table 1) to provide single (SP), double (DP) and triple (TP) pollen combinations. A balanced combination of pollen, though attempted, could not succeed mainly due to problems of synchronisation of flowering in males and females. However the combinations attempted did provide information on pollen effects.

The 14 female and 12 pollen parents were mated in a line X tester system (Kempthorne 1957). The genetic history of the parents would make it clear that they were open-pollinated and could be homo- or heterozygotes. Hence for effecting crosses, one or two phenotypically similar plants representing the modal phenotype of the line were chosen as female parents and pollen used was from plants chosen with similar restrictions. This would only ensure that the genotype of each female or male remained the same (homo- or heterozygote) in all crosses. In other words, though each parent was not highly inbred, they were so selected so as to be of a single genotype. This method is in sharp contrast to the one usually adopted by breeders in which several female and pollen plants would be used for each cross and resulting seeds bulked. The methods employed in this study were found adequate for a relative evaluation.

Stickiness of pollen grains, mechanical damage during brushing them off to a petri dish and inevitable unequal amounts of pollen mixture dissuaded us from using mixed bulk pollen in effecting mucromphs. It was found easier to dust fresh pollen from anthers on to stigma divided tentatively into two or three equal portions to provide DP and TP. This method resulted in good seed set and pollen competition at the junctions of partitions.

Out of the 168 mucromphs, one cross – POLR X TIP3 – failed mainly due to the non-availability of healthy buds in the female parent at the time of pollination. The F1 of the 167 crosses was grown in a randomised blocks design during 1976 in two replications. Each row was of 3 m length with a spacing of 75 cm between rows and 10 cm between plants.

Character Studied

Observations recorded on 'plant characters' included (i) plant height (HT), (ii) number of primary branches (PB), (iii) number of secondary branches (SB), (iv) length of primary branch (LP) in cm at about mid-height of a plant, (v) total number of siliquae (including those on the secondary branches) – (SQ), (vi) seed yield in gm – (YP) on the primary branch mentioned in (iv), and (vii) single plant yield in gm – (YD). Since the primary branch at mid-height of the plant can be considered to represent approximately the mean physiological age of all primary branches of the plant, observations were recorded on it in preference to the main axis chosen usually by breeders.

(i) Mean number of days to flowering (MF), (ii) weighted log variance of days to flowering (VF), (iii) bulk yield (BY) and (iv) seedling vigour (SV) were the 'plot characters' that were studied. Dry weight of a sample of seedlings (excluding roots) taken at random from each plot 36 days after sowing was taken as a quantitative measure of seedling vigour (SV).

Flowering time is an important component but is difficult to measure in cultures like mucromphs which contain a number of diverse genotypes. However, it is possible to fit a frequency distribution of flowering. Observations were started 51 days after sowing when plants started flowering. The cumulative frequency of plants that flowered was observed in each plot at 2-day intervals upto 69 days after sowing. This covered the major part of the flowering phase. The last date of flowering in each plot was also noted in order to provide the last class interval. Since the total number of plants was noted in each plot, the plants that remained without flowering until after 69 days were counted in the last class interval. Thus it was possible to find the distribution of flowering in each plot.

Table 1. Parents of mucromphs

Female			Male		
Group	Code	Pedigree	Group	Code	Pedigree
SC	KLIB	KL17 X IB 3^{y} (F2)	SP	BELE	Bele ^{e}
	5995	DS- KL 17 X SS – Pusa BST 2 (F3)		TORP	Torpe ^{e}
	1555	DS-KT 5907 X SS-Assam Local (F3)		DS17	DS 17D
TC	7618	KT 5905 (PV X OR) X I 84-63 (F2)		PUKA	Pusa Kalyani
	35K1	GBS II L (PV X OR) X K1 (F2)	DP	BEDS	BELE + DS 17
	73BS	KL17 (OR X PV) X BS182 (F2)		DSPK	DS17 + PUKA
	26K1	GBS II E (PV X OR) X K1 (F2)		BEPK	BELE + PUKA
BP	ID91	IB 6^{y} X DS17D (BIP F2) – 9 – 1		TP71	TORP + 71-6809^{e}
	ID92	IB 6^{y} X DS17D (BIP F2) – 9 – 2	TP	BDP3	BELE + DS17 + PUKA
	ID21	IB 6^{y} X DS17D (BIP F2) – 2 – 1		DP13	DS17 + PUKA + I84-63
	TG73	T1842^{t}X GBS II (BIP F2) – 7 – 3		TLP3	KT 5907 + PUKA + KL17
VT	PUKA	Pusa Kalyani		TIP3	KT 5907 + PUKA + I84-63
	JS41	S–41^{ye}			
	POLR	Polar^{e}			

^{y} Yellow sarson, ^{t} Toria, ^{e} Exotic

SC = Single crosses, DS = Disruptive selection, SS = Stabilising selection, TC = 3-way crosses, BP = Biparental single plant progeny, VT = Varieties, SP = Single pollen, DP = Double pollen, TP = Triple pollen, PV = Productive variant, OR = Original

The parameter, mean flowering time (MF), would then be given by MF = $\Sigma f_i x_i/(\Sigma f_i)$, i = 1 to n, f_i = frequency in and x_i = mid-point of i^{th} class interval.

The variance of flowering time in each plot (VF) is another important character given by VF = $\Sigma f_i (x_i - \bar{x})^2/[(\Sigma f_i) - 1]$

Being of second degree, values of VF were transformed as $K \log_e (L/K)$ where K = $(\Sigma f_i) - 1$ and L = $\Sigma f_i (x_i - \bar{x})^2$ were analysed.

The biological potential of mucromphs was further evaluated by five ratio characters – number of PB per unit height (PBH), number of SB per PB (SPB), number of SQ per unit length of PB (SLP), yield per unit length of PB (YLP) and yield per siliqua (YDS). If n plants were sampled in each plot, the ratio (SPB), for example, can be measured in two ways – (i) $R_\ell = \frac{1}{n} \sum_{i=1}^{n} (y_i/x_i)$, where y_i = number of SB and x_i = number of PB in i^{th} plant. (ii) $R_m = \Sigma y_i/\Sigma x_i$. Breeders usually use (i) in their analysis. The ratios (i) and (ii) are biased estimates. An almost unbiased ratio estimator for y/x is $R_c = (nR_\ell - R_m)/(n - 1)$ (Murthy and Nanjamma 1959). The estimates given by R_c were used for analysis of ratio characters.

Methods of Analysis

As mentioned earlier, there were in all 167 crosses in two replications to give a total of 334 mucromph lines. The aim was to use a fairly large sample in each line and to keep sample sizes equal between lines. Root disease that occurred early in the growth phase, though eventually controlled, resulted in unequal sample sizes. The frequency of these unequal samples was as follows:

	Sample size (Plants)									Total
	3	4	5	6	7	8	9	10	11	
Number of lines (over 2 replications)	9	13	37	28	25	25	28	162	7	334

To avoid loss of information, it was decided to use these unequal sample sizes and also to modify the line X tester analysis to take into account 167 crosses, by reducing 1 d.f. in the male X female interaction s.s. Weighted ANOVA with sample sizes as weights was found appropriate in this context.

Estimates of general (gca) and specific (sca) combining ability effects were calculated after correcting the cell means for the bias arising out of the use of unequal sample sizes as follows:

If n_i is the sample size and x_i, the sample total for character in i^{th} cross (i = 1,2,... N), then its sample mean $m_i = x_i/n_i$ and the mean of m_i = $m = (1/N) \sum_{i=1}^{N} x_i/n_i$. The general mean $M = (\sum_{i=1}^{N} x_i)/(\sum_{i=1}^{N} n_i)$. m = M only when $n_i = n$ for all i or when sample sizes are equal for the crosses. When $n_i \neq n$ for all i there is a bias, given by B = m – M that would upset the constraints $\sum_{i=1}^{p} g_i = \sum_{j=1}^{f} g_j = 0$ and $\sum_i s_{ij} = \sum_j s_{ij} = 0$, where g_i is the gca effect of the i^{th} parent, p, the number of male and f, the number of female parents and s_{ij}, the sca effect of the cross, i X j.

It was therefore necessary to correct for the bias B and obtain the corrected mean, m_{ic} for i^{th} cross. An efficient way was to distribute B over the means of the crosses proportional to the inverse of the respective sample sizes. This is logical since the higher the sample size, the lower would be the bias. Thus $m_{ic} = m_i - NBC$ where $C = (1/n_i)/\sum_{i=1}^{N} (1/n_i)$. Then $m = \frac{\Sigma m_{ic}}{N} = M$, so that B = 0. The corrected means, m_{ic} were used in the analysis of data.

The adjusted gca effects calculated on the corrected means were

$$g_i = [(1/p) (\sum_{j=1}^{p} m_{ij})] - M \text{ and}$$

$$g_j = [(1/f) (\sum_{i=1}^{f} m_{ij})] - M$$

where g_i = gca effect of the i^{th} female parent

g_j = gca effect of the j^{th} male parent

(i = 1, 2, ... f, j = 1, 2, ... p)

m_{ij} = corrected mean for the cross 1 X j and

M = grand mean.

The sca effect (s_{ij}) for the cross (i X j) was estimated as $s_{ij} = m_{ij} - g_i - g_j - M$.

For each cross, (i X j), the quantity ($g_i + g_j + s_{ij}$) was also computed and its significance from zero value tested.

Var ($g_i + g_j + s_{ij}$) = Var (g_i) + Var (g_j) + Var (s_{ij}) since the effects g_i, g_j and s_{ij} are independent and = (1/N) (mf – 1) σ^2 where σ^2 = error m.s. in L X T ANOVA and N = total number of plants in the whole experiment on which observations were made.

Utilisation of Information Provided by Combining Ability Effects

The gca effects of parents and sca effects of crosses were obtained for all the 16 characters based on the line X tester model. Since the character components are correlated either positively or negatively, it is usual to find, for a particular parent, gca in the desirable direction for some characters and in the undesirable direction for others. The problem of ascertaining the status of a parent with respect to gca over a number of component characters assumes importance in this context. Since the emphasis in our study was on a relative assessment of parental potential, the following method was devised.

a) The gca and sca effects were tested for their significance from null value.

b) The parental gca was assessed for each character using a norm m equal to the mean of significant gca effects of parents for that character. The non-significant gca effects were ignored since they were not statistically different from zero. For convenience, those parents whose gca effect was greater than or equal to m were classified as High (H) and assigned a score + 1; others were classified Low (L) and a score –1. It is important to consider not only the magnitude but also the desirable direction of gca effects to classify them as H or L. For example, a parent whose gca effect was less than m for flowering time would be classed H if the breeder was interested in earliness. The parents thus were scored for each character and a final total score was computed for each parent over all the characters. Again the mean of total scores was used as the final norm to classify a parent as H or L over all the characters.

c) The same procedure was adopted to detect whether a cross had H or L sca over all characters. In this case, two contingencies arose – (i) some crosses had an overall zero score due to cancellation of + 1 and –1 scores over the characters and (ii) some re-

ceived the zero score due to non-significant sca effects for each component character. In our study, based on the final norm, a zero total score was classed as H (as the final norm was negative); hence contingency (i) was identified by assigning a status HC (High by cancellation) and (ii) by HN (High by non-significance). The final status with regard to $g_i + g_j + s_{ij}$ was also assigned to each cross in a similar manner.

Defining a Heterotic Cross

Since the parents of mucromphs were genetically complex (including heterozygosity to different degrees), it would not be possible to genetically duplicate them over seasons or replications. So estimating parental mean values by growing them again along with mucromphs was found impracticable. However, as inherent in a line × tester design, a reliable estimate of the parental mean for a relative evaluation was the marginal means, so long as the number of parents was adequate. We therefore chose the marginal means as the best estimates of parental ones for calculating heterosis.

As in the case of gca, the magnitude and direction of heterosis would also vary from character to character. However, a breeder would be interested in a method to decide whether a cross could be considered overall heterotic or not. The following logic was used to find a solution:

a) For every character, a cross was assigned a status B if its mean exceeded that of the superior parent, otherwise a status W. The frequency of B's over all the characters could therefore be counted for every cross.

b) If P1 is the mean of the superior and P2 that of the other parent, M, the value of the midparent and H, the value of the mucromph, four possibilities exist: (i) $H > P1$ (ii) $P1 \geqslant H > M$ (iii) $M \geqslant H \geqslant P2$ (iv) $P2 > H$. When a cross is repeated indefinitely over time or space, it is possible to argue that the apriori probability of each of the events, in particular of (i) = 0.25. When a cross is checked for heterosis over 16 characters, the number of B's it will score will be determined by the binomial distribution, $(\frac{3}{4} + \frac{1}{4})^{16}$, where $pr(B) = \frac{1}{4}$ and $pr(W) = \frac{3}{4}$. The mean of this distribution = $16 \times \frac{1}{4} = 4$. This was chosen as the norm and thus mucromphs which scored 4 or more B's were taken to be overall heterotic.

Heterosis in Relation to Combining Ability

All the mucromphs could be classified under HH, HL and LL groups where, for example, HL would mean that one parent was H and the other L in their overall gca. Thus 28, 84 and 55 mucromphs were assigned to the classes HH, HL and LL respectively, out of which 22, 58 and 36 were heterotic. A breeder would be tempted to observe that a proportion 22/28 was heterotic in HH, 58/84 in HL and 36/55 in LL. We were interested in answering the question: given a heterotic cross, what are the chances for it to be found in HH, HL or LL? The answer is of direct relevance to formulating a repeatable breeding strategy since then one would be able to place more weight on making crosses of the type HH, HL or LL, where the chances for heterosis were found to be high. There were in all 116 heterotic crosses of which 22 were found in HH, 58 in HL and 36 in LL, implying that the chances for a heterotic cross to be found in HL were high. This could be proved. In general, let there be n classes, $K_1, K_2, \ldots K_n$, based on parental gca. Let the class K_i have C_i crosses of which h_i are heterotic.

Let $\sum_{i=1}^{n} C_i = C$ and $\sum_{i=1}^{n} h_i = H$. Let E be the event that a given cross is heterotic. We then require the probability that X belongs to the class K_j (say) given E has occurred, i.e., we require $Pr[(X \epsilon K_j)|E]$.

Now $Pr(X \epsilon K_j)$ or, for short, $Pr(K_j) = C_j/C$, so that $\sum_{j=1}^{n} Pr(X \epsilon K_j) = 1$.

$Pr(E|K_j) = h_j/C_j$, where we assume that the observed proportion h_j/C_j will represent the conditional probability (this is the best estimate in any case).

Thus the required probability, $Pr[(X \epsilon K_j)|E]$ or, for short, $Pr(K_j|E)$ will be, according to Bayes' theorem,

$$Pr(K_j|E) = \frac{Pr(E|K_j).\,Pr(K_j)}{\sum_{j=1}^{n} Pr(E|K_j).\,Pr(K_j)} = \frac{h_j/C_j \cdot C_j/C}{\sum_{j=1}^{n} h_j/C}$$

$$= \frac{h_j}{\sum_{j=1}^{n} h_j} = \frac{h_j}{H} = \frac{\text{number of heterotic crosses in class } K_j}{\text{total number of heterotic crosses.}}$$

It is easy to verify that $\sum_{j=1}^{n} Pr(K_j|E) = 1$.

Thus, we see that the number of crosses made in each group like HH, HL or LL assumes little importance (in estimating the conditional probability, $Pr(E|K_j)$ only) in answering the practical question we formulated earlier. A similar classification of heterotic crosses with respect to their sca and parental gca was helpful to formulate suitable breeding plans and will be discussed in later parts of this series of papers.

Effects of Pollen Combinations

One of the aims of using multiple pollen was to detect the main and interacting effects of pollen combinations as reflected in the measurements of quantitative characters. Though a balanced design of pollen combinations could not be adhered to as explained earlier, the following pollen effects could be computed from the 12 pollen combinations.

I. Pollen interaction effect of BEDS = gca of BEDS − (gca of BELE + gca of DS17)

II. Pollen interaction effect of DSPK = gca of DSPK − (gca of DS17 + gca of PUKA)

III. Pollen interaction effect of BEPK = gca of BEPK − (gca of PUKA + gca of BELE)

IV. Pollen interaction effect of BDP3 = gca of BDP3 − (gca of BELE + gca of PUKA + gca of DS17 + I + II + III)

V. Pollen main effect of 71–6809 + Pollen interaction effect of TP71 = gca of TP71 − gca of TORP

VI. Pollen main effect of I 84–63 + Pollen interaction effect of DS17 × I 84–63 + Pollen interaction effect of PUKA × I 84 − 63 + Pollen interaction effect of DPI3 = gca of DPI3 − gca of DSPK

VII. All possible pollen main and interaction effects in pollen combination TLP3 with the pollen main effect of PUKA eliminated = gca of TLP3 − gca of BEDS

VIII. All possible pollen main effects and interaction effects in pollen combination TIP3 except pollen main effect of PUKA = gca of TIP3 − gca of PUKA

Discussion

An appropriate mating system and optimum selection procedure are two vital components of scientific plant breed-

ing. Theoretical results developed on a particular genetic choice of parents are hardly applicable to practical situations since it is hard to identify an individual genotype or a group of them from their phenotypes. However, a breeder has necessarily to depend on phenotypic measurements. It is therefore relevant to devise repeatable methods to draw pertinent inferences for a relative evaluation of parents and hybrids. These have been described in the preceding pages.

The crop plant we have chosen as the experimental material has permitted a trial with a highly complex mating system. The mating design we chose was the conventional line × tester, but we modified the restrictions on parents to enable the use of partially inbred or open-pollinated plants as parents. This relaxation, while avoiding the effects of inbreeding depression in cross-pollinated crops such as turnip rape, would reflect on the precision of the analysis. That was the reason why only a relative evaluation of the parental or hybrid potential was kept as our major objective. As a rule, the results would be used only to plan the next phase of breeding.

The F1 generation of hybrids formed the test material. The gca and sca components would define the genetic properties of a set of parental and hybrid genotypes as exhibited by the phenotypic values of a metric character. Such genetic effects manifested over a number of relevant and minor characters representing the entire growth phase of a plant were assessed by repeatable methods to finally characterise the genetic status of a parent as H or L. Thus, the methods advocated were quite sound and of more practical relevance than those which would aim to estimating the genetic effects based on a single or two–gene diallelic model. They also avoid the need to test the fit of the data to a one or two–gene model in an attempt to extend the theoretical results available on them.

In a similar manner, the method developed to identify a heterotic cross based on the magnitude of desirable heterosis over a number of component characters was oriented to practical applications. The relation between gca, sca and heterosis (to be reported later) confirmed the value of these methods.

Even when an equal number of H and L parents were involved in a diallel, it would not be possible to have equal number of crosses under the parental gca groups, HH, HL, LH and LL, due to the inevitable inclusion of parents in the diallel crosses. If, in addition, the H and L parents occur with unequal frequency, the discrepancies would be more pronounced. The simple proportion of heterotic crosses observed in each group could not, in the circumstances, reflect the true picture. Hence we chose to ask the question, given a heterotic cross, what was the probability that it would be found in HH, HL or LL group?

The large number of mucromphs generated by multiple pollen on multiple crosses provided an apt system to test the utility and power of the methods propounded here, the results of which will follow as the next part of this paper.

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